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Development of comprehensive river typology based on macrophytes in the mountain-lowland gradient of different Central European ecoregions

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Abstract The aim of the study was to identify the vegetation pattern in the different types of watercourses basing on survey in reference conditions in a wide geographical gradient, including mountain, upland and lowland rivers. We tested relationship between composition of macrophytes to environmental variables including: altitude, slope, catchment area, geology of valley, land use, hydromorphological features, water physical and chemical measurements. Analysis based on 109 pristine river sites located throughout major types of rivers in Central Europe. Qualitative and quantitative plant surveys were carried out between 2005 and 2013. Based on TWINSpan classification and DCA analysis, six macrophyte types were

distinguished. The lowland sites were divided into the following three types: humic rivers and two types of siliceous rivers depending on the catchment area, including medium-large and small rivers. The mountain and upland rivers were divided into three geological types: siliceous, calcareous and gravel. We found that the variation of macrophyte communities was determined by several habitat factors (mainly altitude, flow type, riverbed granulometry, conductivity and alkalinity), whereas the spatial factor was rather limited; further, the plant diversity was not reflected accurately by the European ecoregion approach.

Keywords Aquatic plants · River typology · Reference condition · Undisturbed conditions · Water Framework Directive

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Introduction

The EU Water Framework Directive, adopted in 2000, provided a framework for the EU water policy (the European Commission, 2000). It introduced a new approach to the assessment and classification of surface waters, which is based on biological components such as macrophytes, phytoplankton, benthic macroinvertebrates and fish. To accurately assess the ecology of a water body, the description of the actual state of these organism groups should be compared with their development in an undisturbed reference situation. Therefore, for each surface water body type,

type-specific biological reference conditions must be established. Thus, the identification of biological attributes developing under reference conditions as well as the refinement of the freshwater typology is key issues in effective ecological classification systems, and more generally, a principle of water monitoring and water policy in the EU (Nöges et al., 2009; Mao & Richards, 2012).

Reference conditions represent a pristine state or nearly state of certain types of aquatic ecosystems in the absence of human disturbance or alteration (e.g., Nijboer et al., 2004; Stoddard et al., 2006; Baattrup-Pedersen et al., 2008, 2009; Sánchez-Montoya et al., 2009; Yates & Bailey, 2010). In the case of rivers, this state of condition is almost absent or rare today because river ecosystems are among the most degraded components of the biosphere. The human-generated impact on rivers causes a variety of problems, including eutrophication, enrichment of organic pollutants, acidification and changes in hydrology and morphology (Malmqvist & Rundle, 2002; Tombolini et al., 2014). Moreover, several groups of aquatic organisms suffer from fisheries and losses of river integrity. In Central Europe, we have been destroying and polluting rivers for many decades; therefore, it is particularly difficult to locate pristine conditions.

Two different approaches are commonly used to classify the different typologies of rivers (Ferréol et al., 2005). In the ‘top-down’ approach, the classification is based on large-scale environmental attributes as an ecoregion, geology or altitude (Haslam, 1978). In the ‘bottom-up’ approach, the analysis of aquatic communities is used to classify the rivers (Holmes, 1983). In the present study, a comprehensive typology of the entire range of Polish rivers is defined with the bottom-up approach using the macrophyte assemblage.

The ecological response of macrophytes is a complex issue due to the interrelation of certain environmental factors, which makes bioindication for single factors difficult at the species level (see Wiegand, 1984; Demars & Edwards, 2009; Steffena et al., 2014). Nevertheless, macrophytes were introduced into freshwater monitoring as indicators of river degradation, especially of more persistent and constant habitat changes, which can integrate the long-term effects of disturbances (Westlake, 1975; Smolders et al., 2001; Ceschin et al., 2010). Macrophyte-based methods focus mainly on the determination of eutrophication (e.g., Holmes et al., 1999; Meilinger et al., 2005; Haury et al., 2006; Szoszkiewicz et al., 2006, 2010b; Schneider, 2007; Willby et al., 2009;

Gebler et al., 2014) or acidification (Trempe & Kohler, 1995; O’Hare et al., 2006). However, macrophyte methods are also used to assess river degradation in a more holistic or integrative way (Passauer et al., 2002; Schaumburg et al., 2004). The importance of macrophytes in biological river assessment is formally recognised under the Water Framework Directive (European Commission, 2000). Indeed, this group of organisms is an essential element in the monitoring of ecological status and surface water quality.

Based on a large dataset from four different ecoregions, the present study aims to compare the reference conditions in a wide geographical gradient, including mountain, upland and lowland rivers. Several studies have previously been conducted on macrophytes at the pristine sites; however, they were limited to lowland sites (Baattrup-Pedersen et al., 2008; Birk & Willby, 2010; Szoszkiewicz et al., 2010a), and the representation of non-lowland sites was negligible (Baattrup-Pedersen et al., 2006). Moreover, the typological system of river macrophytes was verified. We anticipate that the aquatic vegetation in the reference rivers is significantly diversified due to various environmental factors such as water chemistry, hydromorphology and geology. We hypothesise that macrophyte-based river types reflect specific abiotic habitats, which can be accurately revealed in undisturbed conditions.

Materials and methods

Site selection

The study was based on a countrywide survey conducted in Poland with a dataset of 1,100 sites located on 450 watercourses. The database was compiled between 2005 and 2013, and field surveys were conducted personally (or at least assisted) by the authors of this paper. The surveyed rivers covered the entire area of Poland, and much was accomplished by reaching every potential reference site. All the existing river types were investigated during the surveys (Journal of Laws, 2011b). Criteria and results concerning the selection of reference sites are described in the “Results” section.

Macrophyte surveys

Macrophyte surveys were conducted during the summer seasons of 2005–2013, between mid-June and

Table 1 Variables recorded in the environmental database

Parameter	Units	Abbreviation
Altitude	m a.s.l.	altit
Slope	‰	slope
Catchment area	km ²	area
Catchment land use		
Forest	%	forest
Wetland	%	wetl
Freshwater	%	fresh
Grassland	%	grass
Arable land	%	arabl
Urban areas	%	urban
Hydromorphological metrics		
Habitat Quality Assessment	Quantitative (0–100)	HQA
Habitat Modification Score	Quantitative (0–100)	HMS
Granulometry		
Granulometry index	Quantitative (1–6)	GM _{index}
Bedrock	%	BE
Boulder	%	BO
Cobble	%	CO
Gravel/pebble	%	GP
Sand	%	SA
Silt	%	SI
Peat	%	PE
Flow type		
Flow type index	Quantitative (1–6)	FT _{index}
Free fall	%	FF
Chute	%	CH
Broken standing waves	%	BW
Unbroken standing waves	%	UW
Rippled	%	RP
Smooth	%	SM
No perceptible flow	%	NP
Physical and chemical parameters of water		
pH	–	pH
Alkalinity	mg CaCO ₃ l ⁻¹	alkal
Conductivity	μS cm ⁻¹	cond
Phosphate	mg P l ⁻¹	PO4
Total phosphorus	mg P l ⁻¹	TP
Nitrate nitrogen	mg N l ⁻¹	N-NO3
Ammonium nitrogen	mg N l ⁻¹	N-NH4

mid-September. Field surveys were conducted using the macrophyte method for river assessment (MMOR) (Szoszkiewicz et al., 2012). This method currently

represents the official monitoring approach for rivers in Poland (Journal of Laws, 2011a). The macrophyte survey was conducted along river reaches of 100 m long. Only river macrophytes growing in the water were recorded. The survey includes a list of species and estimated vegetation cover. The presence of each species was recorded with their percentage cover using the following nine-point scale: <0.1, 0.1–1, 1–2.5, 2.5–5, 5–10, 10–25, 25–50, 50–75 and >75%.

Based on the collected data, the total vegetation cover was estimated for each surveyed section. Moreover, the contribution of various growth forms of plants was analysed using seven categories: macroalgae, aquatic liverworts, aquatic mosses, vascular submerged plants (elodeids), vascular submerged plants with floating leaves (nymphoids), vascular free floating plants (pleustophytes) and vascular emergent plants (helophytes).

Environmental surveys

Each survey, in addition to each macrophyte assessment, was supplemented with a complex suite of environmental parameters (Table 1). They included the following: altitude, slope, catchment area, geology of valley, land use, hydromorphological data, water physical and chemical measurements.

Data on the catchment area and the land use of watersheds were derived from the GIS database built for CORINE Land Cover (CLC-2006). The land use is presented as a vector layer. The land use was analysed in the catchment area stretching from a source to a survey site. The following six different groups of land use were distinguished: urban areas, arable lands, grasslands, forests, wetlands and freshwater ecosystems (Table 1).

The hydromorphological evaluation was conducted at each site according to the river habitat survey (RHS) method (Environment Agency, 2003; Szoszkiewicz et al., 2012). RHS gathers data from 500-m stretches of rivers. The RHS survey is performed in ten profiles (spotchecks), which are distributed at 50-m intervals. The macrophyte survey section was located inside each of the RHS sites, always between the 6th and the 8th spotcheck. Two numerical metrics based on the RHS protocol were produced (Raven et al., 1998; Szoszkiewicz et al., 2012). High values of the habitat quality assessment (HQA) indicate an extensive presence of a number of natural river features and

high landscape diversity along a river. High values of the habitat modification score (HMS) indicate an extensive anthropogenic alteration, such as bank and channel resectioning, reinforcement or other river engineering construction projects. Furthermore, the percentage of granulometry and flow types were derived from the RHS database. Seven flow types and seven types of riverbed material were distinguished (Table 1). We also calculated the granulometry index (GM_{index}) and the flow type index (FT_{index}).

The granulometry index (GM_{index}) reflects the average grain size composition of the riverbed associated with the kinetic energy of the flow. It is based on the parameter “dominant channel substrate in spotchecks” with the RHS method (section E). Numerical values are assigned to each type of bed material. High values indicate coarse bed material with a diameter ≥ 256 mm (bedrock, boulders), whereas low values indicate fine material with a diameter < 0.06 mm (silt) and organic material (peat).

$$GM_{\text{index}} = \frac{(BE \cdot 6) + (BO \cdot 5) + (CO \cdot 4) + (GP \cdot 3) + (SA \cdot 2) + (SI + PE \cdot 1)}{10},$$

where BE the number of spotchecks with bedrock, BO the number of spotchecks with boulders ($\Phi \geq 256$ mm), CO the number of spotchecks with cobbles ($\Phi = 64\text{--}256$ mm), GP the number of spotchecks with gravel/pebbles ($\Phi = 2\text{--}64$ mm), SA the number of spotchecks with sand ($\Phi = 0.06\text{--}2$ mm), SI the number of spotchecks with silt ($\Phi < 0.06$ mm), and PE the number of spotchecks with peat.

The flow type index (FT_{index}) reflects the average riverbed hydraulic characteristics associated with parameters such as slope, flow velocity and depth. It is based on the parameter “dominant flow type in spotchecks” with the RHS method (section E). Numerical values are assigned to each flow type. High values indicate a turbulent flow of high velocity (free fall/chute and broken standing waves), whereas low values indicate laminar flow (smooth) and no flow (no perceptible flow).

where FF the number of spotchecks with free fall, CH the number of spotchecks with chute, BW the number of spotchecks with broken standing waves, UW the number of spotchecks with unbroken standing waves, RP the number of spotchecks with rippled, SM the number of spotchecks with smooth, and NP the number of spotchecks with no perceptible flow.

Water samples for physical and chemical analyses were typically collected during the same visit, when plant and hydromorphological surveys were performed. Samples were not collected during rainy weather or periods with high runoff; if necessary, an additional visit was organised to collect a water sample. Surface water samples were collected in the midstream below the surface. All samples were filtered using Sartorius cellulose filters with a nominal pore size of $0.45 \mu\text{m}$, except for those used for the determination of total phosphorus. Water samples were cooled and analysed in a laboratory within a 12-h period. The analyses included seven parameters (Table 1, physical and chemical

parameters of water). Electrical conductivity and pH were measured by digital potentiometers (Elmetron CP-401, CC-551). Alkalinity was measured with sulphuric acid to an end point of pH 4.5 in the presence of methyl orange. Concentrations of phosphate (molybdenum blue method), total phosphorus (molybdenum blue method after microwave mineralisation in MARS 5X), nitrate nitrogen (cadmium reduction method) and ammonium nitrogen (Nessler’s method) were determined using a spectrophotometer HACH-LANGE DR/2800.

Statistical procedures

To classify plant data, TWINSpan two-way indicator species analysis (Hill, 1979) was used. This method is a multivariate ordination technique used to classify species and samples. The output of the analysis is a

$$FT_{\text{index}} = \frac{(FF + CH \cdot 6) + (BW \cdot 5) + (UW \cdot 4) + (RP \cdot 3) + (SM \cdot 2) + (NP \cdot 1)}{10},$$

two-way ordered table of species occurrence based on multi-level, two-way partitioning of the correspondence analysis scores. The analysis was performed with PISCES Community Analysis Package 2.0. The major TWINSpan end clusters were defined as macrophyte river types considering their correspondence to the abiotic river typology for Poland. Moreover, river types were distinguished based on the macrophytes of reference sites using detrended correspondence analysis (DCA) from CANOCO for Windows version 4.5 (Ter Braak & Šmilauer, 2002). Rare taxa found at a maximum of 3 sampling sites were excluded from the analysis.

Canonical ordination analysis for relating the composition of macrophytes to environmental variables (physical and chemical parameters of water and hydromorphological features) was carried out using CANOCO 4.5 (Ter Braak and Šmilauer, 2002). The appropriate type of analysis (DCCA) was selected to analyse the biological data using DCA and the length of the gradient, as well as the presence of arch effect (Hill & Gauch, 1980). Preliminary DCA on the biological data revealed that the gradient length was more than four SD (the standard deviation), indicating that the biological data exhibited unimodal responses to underlying environmental variables; this result justified the use of Gaussian multivariate methods. Therefore, a unimodal direct ordination DCCA with a forward variable selection was used to reduce the large set of environmental variables. The statistical significance of the relationship between macrophyte data and environmental parameters was evaluated using the Monte Carlo permutation test (499 permutations) (Gill, 2007).

The analyses of environmental databases began by testing the distribution of water physical and chemical parameters in groups using the W-value according to Shapiro–Wilk criteria (Shapiro & Wilk, 1965). To normalise the distribution, most of the variables were transformed using the Box–Cox transformation (Box & Cox, 1964). The significance of differences in water physical and chemical parameters among macrophyte types of rivers was tested using one-way ANOVA (StatSoft, 2011) together with a Spjøtvoll–Stoline a posteriori test (Sokal & Rohlf, 1995). Brown–Forsyth (1974) and Levene’s tests (1960) were used to assess the equality of variances. The significance of differences in the growth forms of plants among macrophyte types of rivers was tested using non-parametric

Kruskal–Wallis one-way ANOVA (Kruskal & Wallis, 1952; StatSoft, 2011).

Results

Selection of reference sites

The reference site selection was based on four criteria: (1) catchment land use, (2) hydromorphological conditions, (3) physical and chemical parameters of water, and (4) nature protection. Regarding the catchment land use, >60% of forests, wetlands or extensive grasslands, <25% of arable land and <1% of urban area were required for the reference sites. Moreover, exclusively natural and semi-natural forms of land use were restricted to the nearest vicinity of the selected reference river sites (250 m upstream and downstream) as a strip of 50 m from the bank top. These were forests, wetlands, extensive grasslands, and in high mountains—shrubs of *Pinus mugo* and rock debris. The reference hydromorphological conditions were characterised by a significant heterogeneity of the channel morphological structure according to the RHS criteria ($HQA \geq 45$), a lack of anthropogenic alteration of river systems and the absence of any water structures ($HMS \leq 5$, most often $HMS = 0$). Concerning the physical and chemical parameters of water, a low nutrient concentration was required: reactive phosphorus $<0.1 \text{ mg PI}^{-1}$, total phosphorus $<0.2 \text{ mg PI}^{-1}$, nitrate nitrogen $<1.0 \text{ mg NI}^{-1}$, ammonium nitrogen $<0.2 \text{ mg NI}^{-1}$, conductivity $<0.7 \text{ mS cm}^{-1}$ and a lack of anthropogenic acidification and salinisation. Most of the parameters correspond to a very good (1st class) physical and chemical status of water (for reactive phosphorus—good) according to the official Polish standards (Journal of Laws, 2011a).

The requirements concerning the contribution of protected areas were very demanding, and the selected sites were located in the most strictly protected parts of Poland, i.e., World Biosphere Reserves, National Parks, Nature Reserves, Landscape Parks, and Natural Parks, as well as Special Protection Areas under the Birds Directive (European Commission, 1979) and Special Areas of Conservation under the Habitats Directive (European Commission, 1992). Several sites were located in areas subjected to numerous forms of nature conservation, e.g., the Roztoka River is protected as the Tatra Biosphere Reserve, the Tatra National Park, the

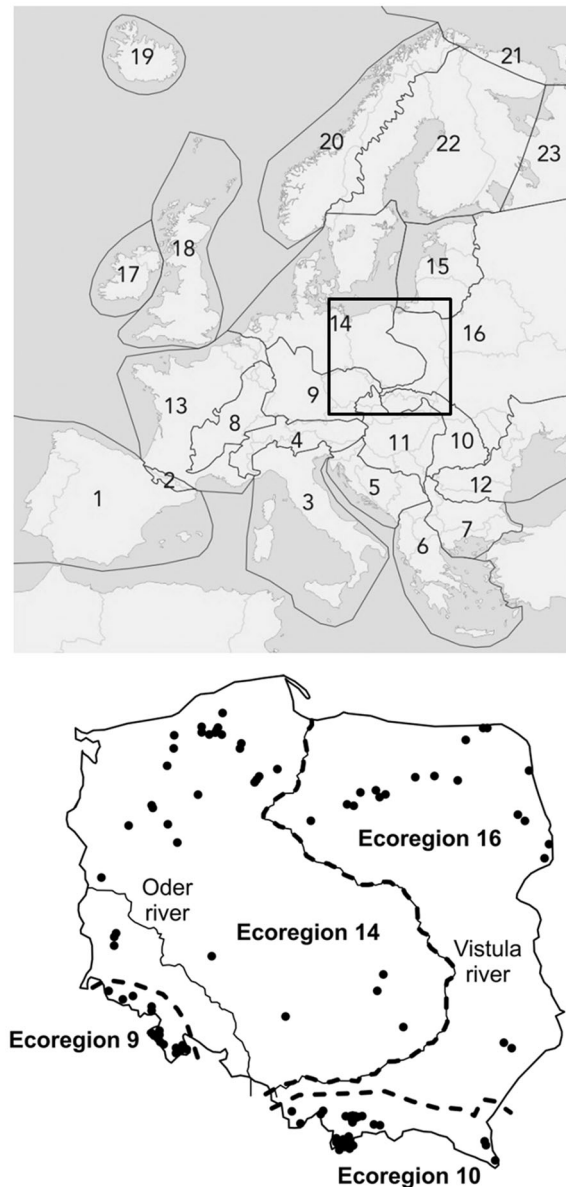


Fig. 1 Distribution of the reference sites of the background ecoregions (WFD, Annex XI, p. 71) Full name of ecoregions: 9 Central Highlands, 10 the Carpathians, 14 Central Plains, 16 Eastern Plains

Tatra Special Protection Area and the Tatra Special Areas of Conservation (PLC120001).

The reference site selection process yielded 109 locations distributed throughout the four ecoregions found in Poland (Fig. 1). The lowland river sites were located in ecoregions 14 (Central Plains) and 16 (Eastern Plains), and the upland and mountain sites

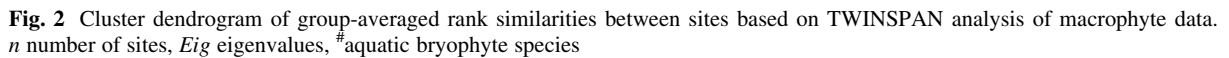
were located in ecoregions 9 (Central Highlands) and 10 (the Carpathians).

Identification of macrophyte river types

The TWINSpan cluster analysis of macrophytes recorded at the selected reference sites resulted in a clear separation of the analysed sites (Fig. 2). The first dichotomy resulted in a clear split (eigenvalues = 0.761), which separated the lowland rivers (45 sites) from the mountain rivers and upland streams (64 sites). The lowland subset was identified by the presence of *Lemna minor*, *Mentha aquatica*, *Sparganium emersum*; the mountains-upland subset was identified by the presence of *Scapania undulata*. The second division of the lowland sites separated 11 humic rivers from others (M-PL1, eigenvalues = 0.487), characterised by the presence of *Carex rostrata*, *Hydrocharis morsus-ranae*, *Lemna minor*, *Nuphar lutea* and *Phragmites australis*. The next division separated the other lowland river subset into two clusters (eigenvalues = 0.425): one with large and medium size catchments (>100 km², 19 sites) and the second one with small size catchments (<100 km², 15 sites). The key species in the medium and large rivers (M-PL2) included *Callitriche cophocarpa*, *Phalaris arundinacea* and *Sparganium emersum*, whereas *Cratoneuron filicinum* and *Veronica beccabunga* were representative species in the small streams (M-PL3).

The second division of mountain and upland rivers separated 19 typical siliceous sites with a stony substrate (M-PL6) from the others (eigenvalues = 0.567), characterised by the presence of *Scapania undulata* and the absence of *Brachythecium rivulare* and *Platyhypnidium riparioides*. The next division separated 9 transitional siliceous/calcareous sites (M-PL5a, eigenvalues = 0.351). The last division separated the subset of other mountain and upland rivers into two clusters (eigenvalues = 0.318): one with a typical calcareous stony substrate (25 sites) and the other one with a gravel substrate (11 sites). The key species in the calcareous rivers (M-PL5) included *Blindia acuta*, *Dichodontium pellucidum* and *Palustriella commutata*, whereas the gravel streams (M-PL4) included *Fontinalis antipyretica*.

The differentiation of river types based on macrophytes from the reference sites tested with the DCA (Fig. 3a) was consistent with the results of TWINSpan cluster analysis (Fig. 2). Groups from the TWINSpan analysis, representing the macrophyte

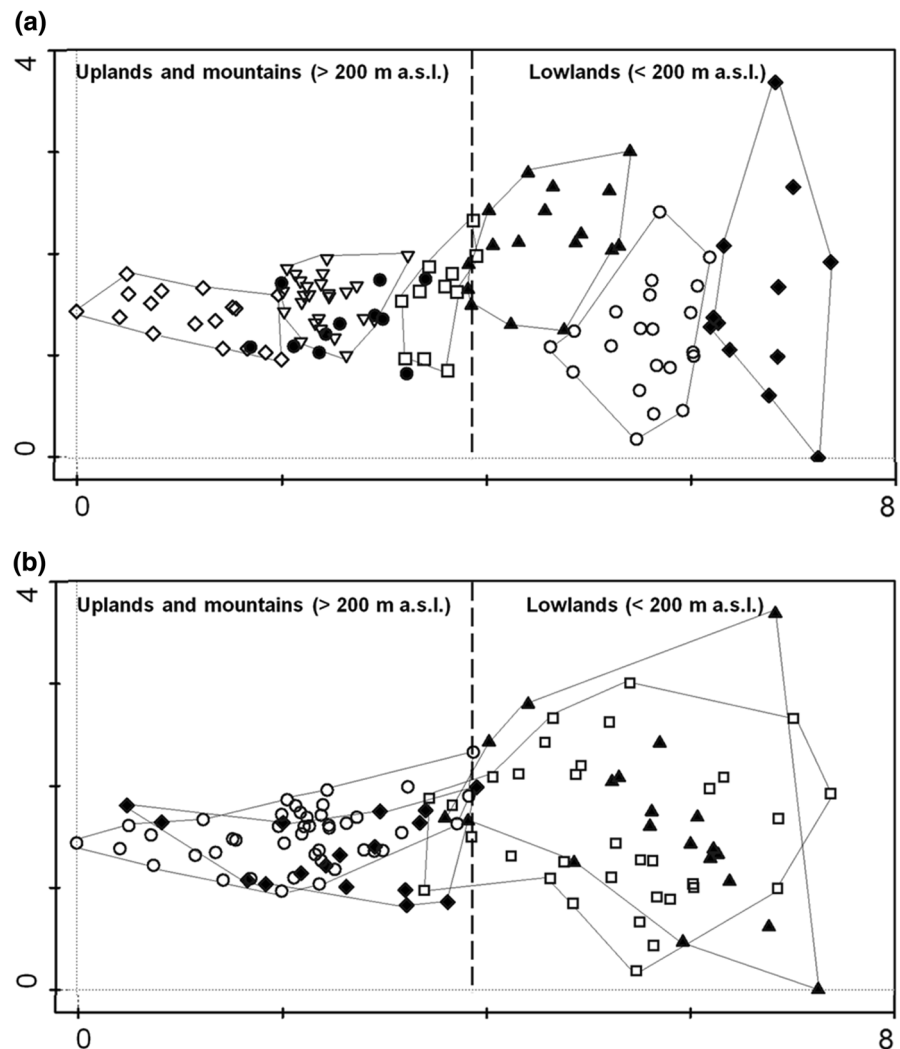


Based on the conducted statistical analyses (TWINSPAN classification and DCA), we distinguished six macrophyte types in the Polish rivers (Figs. 2, 3). The lowland sites were divided into three types, i.e., humic rivers (M-PL1) and two types differentiated in terms of catchment area: medium-large rivers (M-PL2,

Macrophyte differentiation between river types

A total of 133 macrophyte taxa were identified, including 12 macroalgae, 1 aquatic lichen, 14 liverworts, 28 mosses, 25 elodeids, 5 nympheids, 4 pleustophytes and 48 helophytes. The incidence of individual taxa in the analysed types of rivers is presented in Table 2. The number of taxa at the study sites ranged from 9 ± 5 (M-PL6) to 25 ± 9 (M-PL1), and their total cover ranged from 13.2 ± 19.2 (M-PL4) to 49.9 ± 25.0 (M-PL1). Both parameters were significantly different in each of the analysed types of rivers. In general, the upland and mountain macrophyte types were characterised by a smaller number of taxa and smaller total cover compared to the lowland types (Table 2).

Fig. 3 DCA ordination diagrams of the river types based on macrophyte species data of reference sites ($\lambda_1 = 0.801$, $\lambda_2 = 0.283$). **a** Marked groups of TWINSpan analysis: *black diamonds* humic lowland (M-PL1), *circle* medium and large lowland (M-PL2), *black triangle* small lowland (M-PL3), *square* gravel upland streams (M-PL4), *down-triangle* calcareous mountain and upland streams (M-PL5), *black circle* transitional between calcareous and siliceous streams, *diamond* siliceous mountain and upland streams (M-PL6). **b** Marked ecoregions: *black diamonds* Central Highlands (9), *circle* the Carpathians (10), *square* Central Plains (14), *black triangle* Eastern Plains (16)



The distinguished macrophyte types significantly differed in the presence of all the analysed groups of macrophytes ($H > 30.89$, $P < 0.0001$) (Fig. 4). The humic lowland rivers (M-PL1) were distinguished by a high percentage of nymphs and pleustophytes (on average ca. 20%), which were not recorded in larger quantities in any other macrophyte types of rivers. In the medium-large lowland rivers (M-PL2), elodeids (on average 50% of the total cover) and helophytes (on average 25% of the total cover) dominated. These two groups of macrophytes also dominated in small lowland streams (M-PL3), although proportions of their contribution were reversed. Aquatic bryophytes significantly dominated in three mountain and upland types of rivers (on average 50–100% of the total

cover). Macrophytes in siliceous mountain and upland streams were represented mostly by liverworts (M-PL6) (Fig. 4b), whereas the calcareous mountain and upland streams were represented by mosses (M-PL5) (Fig. 4c). In the upland and mountain rivers, nymphs were absent among vascular macrophytes (Fig. 4e), elodeids and pleustophytes occurred in small numbers (Fig. 4d, f), and only helophytes occurred with a higher percentage in upland streams with gravel bottoms—up to 20% (Fig. 4g).

The relationship between aquatic plant species and environmental variables at the reference sites was demonstrated by DCCA analysis and presented graphically (Fig. 5). The first axis accounted for 35.1% and the second axis for 12.2% of the total variance in the

Table 2 The occurrence of macrophyte taxa in the analysed types of rivers

Taxon	Abbreviation	M-PL1	M-PL2	M-PL3	M-PL4	M-PL5	M-PL6
Macroalgae							
<i>Batrachospermum</i> sp.	Batasp_		*	*		*	
<i>Chara globularis</i>	Chaglo		*				
<i>Cladophora</i> sp.	Clasp_	*	**	*	**	**	
<i>Hildenbrandia rivularis</i>	Hilriv		**	*	**	*	
<i>Hydrurus</i> sp.	Hydsp_				*	*	
<i>Lemanea fluviatilis</i>	Lemflu						*
<i>Lyngbya</i> sp.	Lynsp_			*		*	
<i>Mougeotia</i> sp.	Mousp_		*			*	*
<i>Phormidium</i> sp.	Phosp_		*	*	**	****	
<i>Spirogyra</i> sp.	Spisp_	*			*	*	
<i>Ulothrix</i> sp.	Ulosp_			*	*	**	
<i>Vaucheria</i> sp.	Vausp_	*	*	*	**	*	
Lichens							
<i>Collema</i> sp.	Colsp_					*	**
Liverworts							
<i>Chiloscyphus pallescens</i>	Chipal			*	*	*	
<i>Chiloscyphus polyanthos</i>	Chipol		*	*	**	****	*
<i>Conocephalum conicum</i>	Concon		*	***	***	****	
<i>Jungermannia</i> sp.	Jungsp					**	***
<i>Marsupella</i> sp.	Marsp_						***
<i>Nardia</i> sp.	Narsca					*	*
<i>Pellia endiviifolia</i>	Pelend		*	**	**	**	*
<i>Pellia epiphylla</i>	Pelepi			*	**	*	**
<i>Plagiochila asplenoides</i>	Plaasp			*	*	**	*
<i>Plagiochila porelloides</i>	Plapor					*	*
<i>Porella cordaeana</i>	Porcor			*		*	
<i>Scapania</i> sp.	Scasp_			*	*	***	*****
Mosses							
<i>Andreaea</i> sp.	Andsp_						**
<i>Blindia acuta</i>	Bliacu					***	**
<i>Brachythecium rivulare</i>	Brariv		*	***	***	***	*
<i>Bryum pseudotriquetrum</i>	Brypse			*		**	*
<i>Calliergon cordifolium</i>	Calcor		*				
<i>Calliergonella cuspidata</i>	Calcus	*					
<i>Codriophorus acicularis</i>	Codaci						**
<i>Codriophorus aquaticus</i>	Codaqu					*	**
<i>Codriophorus fascicularis</i>	Codfas					*	**
<i>Cratoneuron filicinum</i>	Crafil		*	***	*****	****	*
<i>Dichodontium pellucidum</i>	Dicpel			*	*	****	*
<i>Fissidens</i> sp.	Fissp_			*	*	*	
<i>Fontinalis antipyretica</i>	Fonant		****	**	****	*	*
<i>Hygroamblystegium fluviatile</i>	Hygflu			*	**	*	
<i>Hygroamblystegium tenax</i>	Hygten			*	***	*	*

Table 2 continued

Taxon	Abbreviation	M-PL1	M-PL2	M-PL3	M-PL4	M-PL5	M-PL6
<i>Hygrohypnum duriusculum</i>	Hygdur					*	*
<i>Hygrohypnum luridum</i>	Hyglur				*	****	*
<i>Hygrohypnum mole</i>	Hygmol					*	**
<i>Hygrohypnum ochraceum</i>	Hygoch				*	*	***
<i>Leptodictyum riparium</i>	Leprip		*	***	*		*
<i>Palustriella commutata</i>	Palcom					*****	
<i>Philonotis</i> sp.	Phisp_				*	*	*
<i>Platyhypnidium riparioides</i>	Plarip		*	**	*****	***	
<i>Schistidium apocarpum</i>	Schapo			*		**	*
<i>Schistidium rivulare</i>	Schriv					*	*
<i>Sciuro-hypnum plumosum</i>	Sciplu				*	***	**
<i>Sphagnum</i> sp.	Sphsp_	*					*
<i>Thamnobryum alopecurum</i>	Thaalo				*	*	
Elodeids							
<i>Batrachium aquatile</i>	Bataqu		*		*		
<i>Batrachium circinatum</i>	Batcir		*	*			
<i>Batrachium fluitans</i>	Batflu		**		*		
<i>Batrachium peltatum</i>	Batpel		*		*		
<i>Batrachium trichophyllum</i>	Battri	*	**	*			
<i>Callitriche cophocarpa</i>	Calcop	*	***	*			
<i>Callitriche hamulata</i>	Calham		*				
<i>Ceratophyllum demersum</i>	Cerdem	*					
<i>Ceratophyllum submersum</i>	Cersub	*					
<i>Elodea canadensis</i>	Elocan	****	***	*	*		
<i>Hottonia palustris</i>	Hotpal	*	*				
<i>Myriophyllum spicatum</i>	Myrspi	*	*				
<i>Myriophyllum verticillatum</i>	Myrvet	*					
<i>Potamogeton alpinus</i>	Potalp	***	*	*			
<i>Potamogeton berchtoldii</i>	Potber	*	*	*			
<i>Potamogeton crispus</i>	Potcri		**	*			
<i>Potamogeton lucens</i>	Potluc		*	*			
<i>Potamogeton obtusifolius</i>	Potobt	*	*				
<i>Potamogeton pectinatus</i>	Potpec	*	*	*			
<i>Potamogeton perfoliatus</i>	Potper	*	*	*			
<i>Potamogeton pusillus</i>	Potpus		*	*			
<i>Utricularia vulgaris</i>	Utrvul	**					
Nymphheids							
<i>Hydrocharis morsus-ranae</i>	Hydmor	****	*				
<i>Nuphar lutea</i>	Nuplut	****	*				
<i>Nymphaea alba</i>	Nymalb	*					
<i>Potamogeton natans</i>	Potnat	***					
<i>Stratiotes aloides</i>	Stralo	*					

Table 2 continued

Taxon	Abbreviation	M-PL1	M-PL2	M-PL3	M-PL4	M-PL5	M-PL6
Pleustophytes							
<i>Lemna minor</i>	Lemmin	*****	***	***	*		
<i>Lemna trisulca</i>	Lemtri	****	**	*			
<i>Spirodela polyrhiza</i>	Spipol	***	*	*			
Helophytes							
<i>Acorus calamus</i>	Acocal	*					
<i>Alisma plantago-aquatica</i>	Alipla	***	**	*			
<i>Berula erecta</i>	Berere	***	***	***	*		
<i>Butomus umbellatus</i>	Butumb		*				
<i>Calla palustris</i>	Calpas	*					
<i>Carex acutiformis</i>	Caracu	****	**	**			
<i>Carex gracilis</i>	Cargra	**	*	*			
<i>Carex paniculata</i>	Carpan	***	*	*			
<i>Carex pseudocyperus</i>	Carpse	**		*			
<i>Carex riparia</i>	Carrip	*	*				
<i>Carex rostrata</i>	Carros	****	*	*			
<i>Cicuta virosa</i>	Cicvir	***					
<i>Eleocharis palustris</i>	Elepal	**					
<i>Equisetum fluviatile</i>	Equflu	***	*	**			
<i>Equisetum palustre</i>	Equipal	**	**	*	*		*
<i>Glyceria fluitans</i>	Glyflu	**	***	****	***	*	*
<i>Glyceria maxima</i>	Glymax	***	**	*			
<i>Iris pseudacorus</i>	Iripse	*	*	*			
<i>Lycopus europaeus</i>	Lyceur	*	**	**			
<i>Lysimachia thyrsiflora</i>	Lysthy	*	*	*			
<i>Lysimachia vulgaris</i>	Lysvul	*	*	*			
<i>Mentha aquatica</i>	Menaqu	***	***	*****	*	*	*
<i>Menyanthes trifoliata</i>	Mentri	**					
<i>Myosotis palustris</i>	Myopal	***	***	****	*	*	*
<i>Nasturtium officinale</i>	Nasoff		*	*	*	**	*
<i>Oenanthe aquatica</i>	Oenaqu	*	*	*			
<i>Peucedanum palustre</i>	Peupal	*	*				
<i>Phalaris arundinacea</i>	Phaaru	***	****	*	*		
<i>Phragmites australis</i>	Phraus	*****	*	*			
<i>Polygonum amphibium</i>	Polamp	*	*				
<i>Ranunculus flammula</i>	Ranfla		*				
<i>Ranunculus lingua</i>	Ranlin	***		*			
<i>Rorippa amphibia</i>	Roramp	***	**	*			
<i>Rumex hydrolapathum</i>	Rumhyd	**	*	*			
<i>Sagittaria sagittifolia</i>	Sagsag	**	*				
<i>Scrophularia umbrosa</i>	Scrumb	*	**	**	*		
<i>Sium latifolium</i>	Siulat	**	*				
<i>Sparganium emersum</i>	Spaeme	***	****	*			
<i>Sparganium erectum</i>	Spaere	***	***	*			
<i>Sparganium minimum</i>	Spamin	*					

Table 2 continued

Taxon	Abbreviation	M-PL1	M-PL2	M-PL3	M-PL4	M-PL5	M-PL6
<i>Thelypteris palustris</i>	Thepal	*					
<i>Typha latifolia</i>	Typlat	***	*	*			
<i>Veronica anagallis-aquatica</i>	Verana	***	***	**			
<i>Veronica beccabunga</i>	Verbec		**	*****	**	*	*
No. of taxa (mean \pm SD)		25 \pm 9	19 \pm 8	19 \pm 5	15 \pm 5	14 \pm 5	9 \pm 5
Total cover (%) (mean \pm SD)		49.9 \pm 25.0	25.4 \pm 23.2	13.5 \pm 13.9	13.2 \pm 19.2	13.9 \pm 15.6	16.0 \pm 14.5

The percentage of sites in a given river type with taxa occurrence was as follows: * 0–20.0%, ** 20.1–40.0%, *** 40.1–60.0%, **** 60.1–80.0%, ***** 80.1–100%

relationships between the aquatic plant species and macrophyte types of rivers. The interpretation of the first two axes appears to be relatively simple. The first axis can be identified with the kinetic energy of water (current velocity), which is determined by altitude and slope, and directly affects the degree of riverbed material fragmentation and the observed types of flow (Fig. 5). The first axis corresponds to a typological criterion of altitude AMSL from Appendix II of WFD (Directive 2000/60/EC), based on which lowland rivers (<200 m a.s.l.) were separated from the upland and mountain rivers (>200 m a.s.l.). The second axis is connected with geology and separates the siliceous rivers from calcareous upland/mountain rivers and humic lowland rivers from mineral rivers (Fig. 5).

The largest number of specific taxa was recorded in the humic lowland rivers (M-PL1). They were species characteristic of peat bogs and included the following: *Calla palustris*, *Carex paniculata*, *C. pseudocyperus*, *C. rostrata*, *Cicuta virosa*, *Menyanthes trifoliata*, *Sparganium minimum*, *Stratiotes aloides*, *Thelypteris palustris*, and *Calliergonella cuspidate*; in addition, we identified the following aquatic species of lentic waters: *Hydrocharis morsus-ranae*, *Lemna trisulca*, *Nymphaea alba*, *Potamogeton natans* and *Utricularia vulgaris* (Table 2, Fig. 5). We also identified specific flora species in the upland and mountain types of rivers. In the siliceous mountain and upland streams (M-PL6), they were mostly acidophilus liverworts: *Jungermania* sp., *Marsupella* sp., *Pellia epiphylla*, and *Scapania* sp. and mosses: *Andreaea* sp., *Codriophorus aquaticus*, *C. acicularis*, *C. fascicularis*, *Hygrohypnum molle* and *H. ochraceum*. In the calcareous mountain and upland streams (M-PL5), the species were mainly calciphilus taxa, including the following liverworts:

Chiloscyphus polyanthos, *Pellia endiviifolia*, and *Porella cordeana* and mosses: *Cratoneuron filicinum*, *Dichodontium pellucidum*, *Fissidens* sp., *Hygrohypnum luridum*, *Palustriella commutata*, and *Schistidium apocarpum*. The three other macrophyte types (M-PL2, M-PL3, M-PL4) of rivers merge in the DCCA ordination diagram into one cloud, and it is difficult to identify taxa specific to each of them (Fig. 5). Those are mostly vascular species, e.g., *Batrachium fluviatile*, *B. peltatum*, *B. trichophyllum*, *Potamogeton alpinus*, and *Veronica anagallis-aquatica*.

Table 3 presents the correlation among environmental factors in the ordination analysis for frequency and cover data. The Monte Carlo permutation test showed that only 11 of the 34 environmental variables presented significant lambda-1 values ($P < 0.05$), and for conditional variance—only 8 variables presented significant values.

Habitat differentiation between the river types

The proposed river typology for the Polish water-courses considers six river types. The river types were identified according to macrophyte criteria, but they are also confirmed by the habitat differentiation, including physical and chemical parameters of water and hydromorphological elements. The river type characteristics are described below (Table 4).

Significant differences in the water quality between the identified types of rivers were found. The largest difference concerned water alkalinity ($F = 33.61$, $P < 0.0001$) and conductivity ($F = 27.12$, $P < 0.0001$). On the other hand, the smallest differences were found for ammonia nitrogen ($F = 3.80$, $P = 0.0017$) and nitrate nitrogen ($F = 3.91$, $P = 0.0015$).

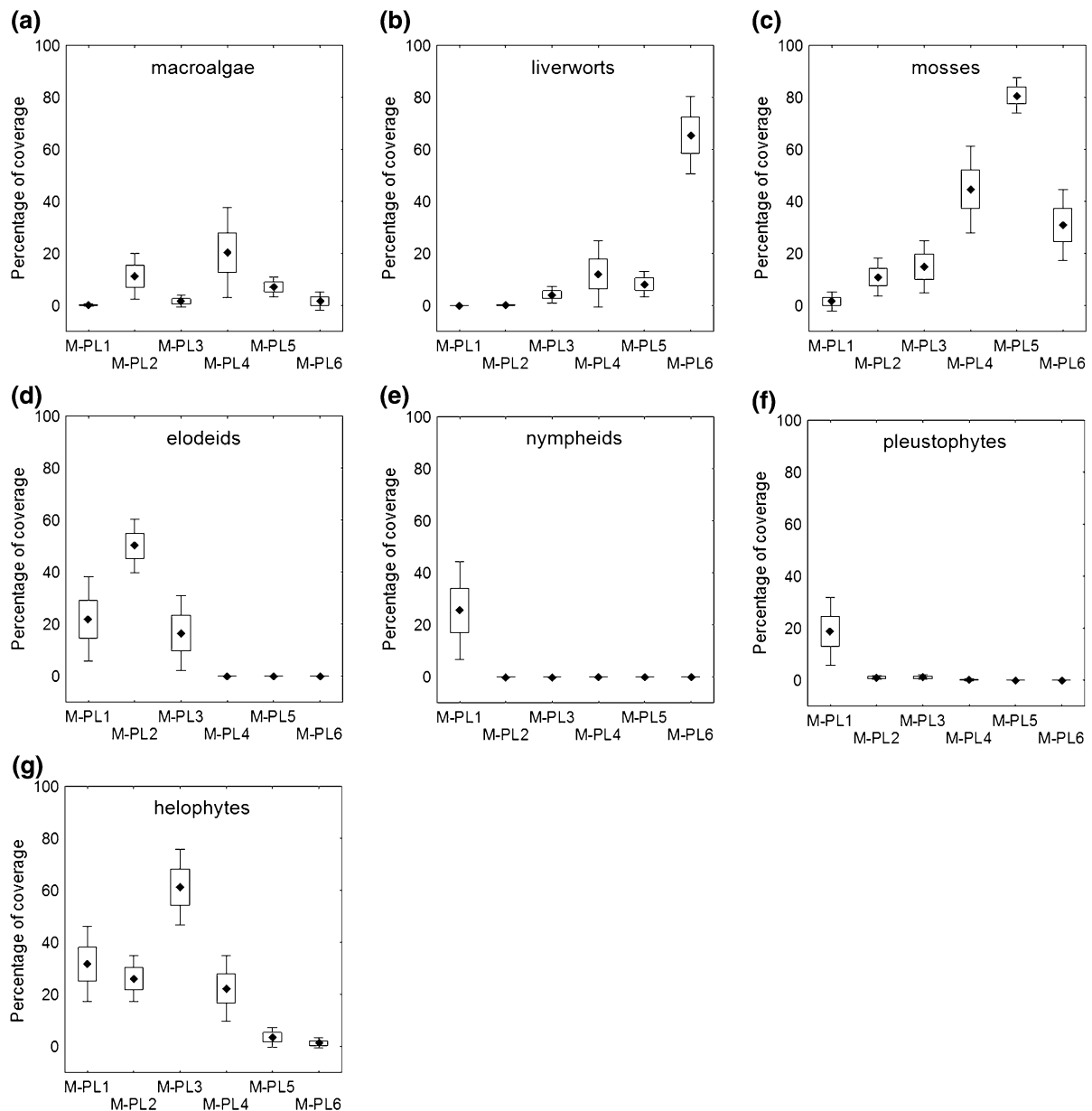


Fig. 4 Percentage of coverage of macrophyte groups (mean \pm SD \pm 95% confidence interval) in separated types of rivers (M-PL1–M-PL6): **a** macroalgae, **b** liverworts, **c** mosses, **d** elodeids, **e** nymphs, **f** pleustophytes, **g** helophytes

Alkalinity and conductivity determined the most important environmental gradient, differentiating the macrophyte diversity to the largest extent. The average values of these parameters decreased gradually from lowland rivers M-PL1–M-PL3 (alkalinity 161.9–176.0 mg $\text{CaCO}_3 \text{ l}^{-1}$, conductivity 446–455 $\mu\text{S cm}^{-1}$) to silicate mountain stream M-PL6 (alkalinity 21.3 mg $\text{CaCO}_3 \text{ l}^{-1}$, conductivity 77 $\mu\text{S cm}^{-1}$). Brown–Forsyth

and Levene’s post-hoc test showed the equality of variances for all the analysed parameters.

The lowland macrophyte river types (M-PL1–M-PL3) were not significantly differentiated in terms of water chemistry (Table 5). The water properties of each lowland river type indicated significant differences compared to the highland and mountain types (M-PL4–M-PL6). The average phosphate

Fig. 5 DCCA ordination diagram of macrophyte species and environmental variables based on reference sites ($\lambda_1 = 0.727$, $\lambda_2 = 0.250$) Full names of metrics are given in Table 1, and full names of macrophytes taxa are given in Table 2

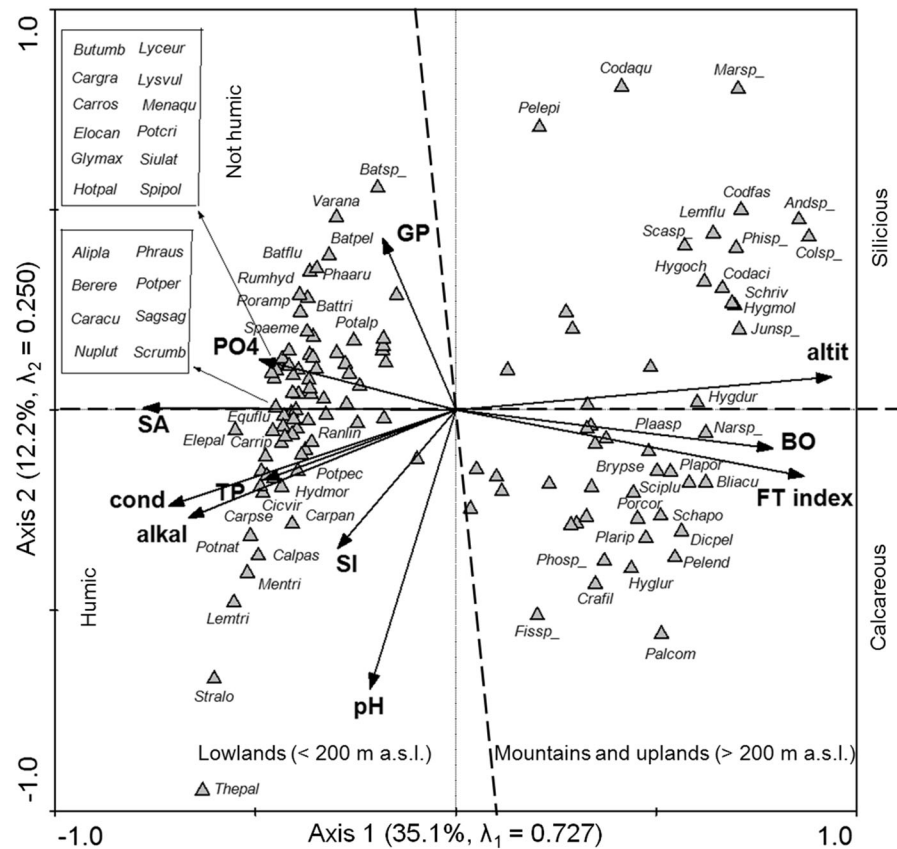


Table 3 Results of the Monte Carlo permutation test of the relationship between species composition and 11 significant environmental variables

Variables	Marginal effects		Conditional effects		
	Lambda1	P	LambdaA	P	F
altit	0.67	0.002	0.67	0.002	8.79
FT _{index}	0.58	0.002	0.09	0.062	1.28
SA	0.49	0.002	0.17	0.002	2.36
BO	0.49	0.002	0.08	0.054	1.29
cond	0.43	0.002	0.09	0.058	1.28
alkal	0.40	0.002	0.11	0.004	1.63
pH	0.29	0.002	0.25	0.002	3.46
TP	0.28	0.002	0.15	0.002	2.11
SI	0.25	0.002	0.21	0.002	2.92
PO ₄	0.25	0.002	0.11	0.004	1.51
GP	0.18	0.002	0.14	0.002	2.02

Lambda-1 is the proportion of variance explained by each single environmental variable, and Lambda-A is the proportion of conditional variance explained by the variable in forward selection. Full names of metrics are given in Table 1

concentrations in the lowland rivers (0.065–0.081 mg PI^{-1}) were 2–3 times higher than in the highland and mountain streams (0.023–0.033 mg PI^{-1}). A similar trend was also found for total phosphate (0.142–0.149 and 0.062–0.091 mg PI^{-1} , respectively). The siliceous mountain and upland rivers (M-PL6) were the most specific type in terms of water chemistry. There was a strong significant difference ($P < 0.001$) compared to all the other types in terms of the pH, alkalinity and conductivity. The average pH of water in the M-PL6 type was 6.92, whereas in the other types of rivers it varied between 7.56 (M-PL1) and 7.95 (M-PL5). Similarly, the lowest alkalinity was observed in the M-PL6 type, i.e., on average 21.3 mg $\text{CaCO}_3 \text{ l}^{-1}$, whereas in the other types of rivers, it ranged between 83.7 (M-PL5) and 176.0 (M-PL1) mg $\text{CaCO}_3 \text{ l}^{-1}$. The situation is similar in the case of conductivity, where the rivers of the M-PL6 type had the lowest value of 77 $\mu\text{S cm}^{-1}$. In the other rivers, the conductivity ranged between 196 (M-PL5) and 455 (M-PL3) $\mu\text{S cm}^{-1}$.

Table 4 Major habitat characteristics of river types

Parameters	Units	River types					
		M-PL1 Humic lowland	M-PL2 Large and medium lowland	M-PL3 Small lowland	M-PL4 Gravelly upland	M-PL5 Calcareous mountain and upland	M-PL6 Siliceous mountain and upland
Altitude	m a.s.l.	119.3 ± 29.4	112.0 ± 58.0	123.1 ± 56.3	426.0 ± 270.7	818.3 ± 186.3	1164.2 ± 456.0
Slope	%	0.9 ± 1.2	1.4 ± 0.9	7.0 ± 7.3	30.3 ± 22.7	95.6 ± 78.4	216.9 ± 178.9
Catchment area	km ²	<1,000	>100	<100	<1,000	<100	<100
Geology of valley	–	Peat river floodplains, areas of peat on outwash plains	Glacial sands and gravels, clay and loess	Glacial sands and gravels, clay and loess	Sandstone, conglomerates, slates, loess and chalk	Carbonate rocks—chalk, limestone, loess, flysch, marl and conglomerates	Igneous and metamorphic rocks— granite, gneiss and slates
HQA	–	54.3 ± 9.1	60.6 ± 6.2	60.4 ± 9.4	64.7 ± 6.9	55.2 ± 8.0	53.6 ± 9.9
HMS	–	3.3 ± 2.9	1.5 ± 2.5	2.5 ± 2.8	3.4 ± 2.6	1.5 ± 2.4	1.0 ± 2.0
Dominant granulometry	% bed	61% SA, 36% SI + PE	56% SA, 39% GP	50% SA, 33% GP	46% GP, 20% CO	47% BO, 32% CO	52% BO, 21% CO
GM _{index}	–	1.7 ± 0.4	2.5 ± 0.4	2.5 ± 0.5	3.4 ± 0.6	4.5 ± 0.6	4.6 ± 0.5
Dominant flow type	% bed	52% SM, 28% RP	55% RP, 34% SM	44% RP, 32% SM	49% RP, 31% UW	49% UW, 26% CH	28% UW, 27% RP, 23 % CH
FT _{index}	–	2.1 ± 0.6	2.7 ± 0.5	3.0 ± 0.8	3.6 ± 0.6	4.4 ± 0.7	4.3 ± 0.8
pH	–	7.56 ± 0.38	7.88 ± 0.23	7.84 ± 0.32	7.94 ± 0.30	7.95 ± 0.28	6.92 ± 0.50
Alkalinity	mg CaCO ₃ l ⁻¹	176.0 ± 40.6	161.9 ± 80.8	168.9 ± 62.4	137.5 ± 97.0	83.7 ± 41.0	21.3 ± 20.5
Conductivity	µS cm ⁻¹	451 ± 175	446 ± 190	455 ± 179	261 ± 124	196 ± 87	77 ± 75
Phosphate	mg P l ⁻¹	0.065 ± 0.051	0.074 ± 0.046	0.081 ± 0.049	0.033 ± 0.046	0.023 ± 0.020	0.031 ± 0.027
Total phosphorus	mg P l ⁻¹	0.148 ± 0.056	0.149 ± 0.053	0.142 ± 0.078	0.091 ± 0.081	0.078 ± 0.075	0.062 ± 0.044
Nitrates	mg N l ⁻¹	0.29 ± 0.33	0.48 ± 0.56	0.25 ± 0.31	0.79 ± 0.38	0.41 ± 0.43	0.44 ± 0.48
Ammonium	mg N l ⁻¹	0.12 ± 0.07	0.11 ± 0.06	0.08 ± 0.08	0.08 ± 0.07	0.08 ± 0.05	0.02 ± 0.03

Altitude, slope, HQA, HMS, GM_{index}, FT_{index}, physical and chemical parameters are given as mean ± SD. Full names of metrics are given in Table 1

Table 5 The significance of differences of physical and chemical parameters among macrophyte river types using a Spjotvoll–Stoline a posteriori test (significance: $P \geq 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

	River types				
	M-PL2	M-PL3	M-PL4	M-PL5	M-PL6
M-PL1	–	–	(N-NO ₃)**	(alkal)*** (cond)*	(alkal, cond)*** (pH, TP, N-NH ₄)*
M-PL2		–	(PO ₄)** (TP)*	(cond, PO ₄ *** (alkal, TP)**	(pH, alkal, cond, PO ₄ , TP)*** (N-NH ₄)**
M-PL3			(PO ₄ , N-NO ₃)** (TP)*	(cond, PO ₄ *** (alkal)**; (TP)*	(pH, alkal, cond, PO ₄)*** (TP)**
M-PL4				–	(pH, alkal, cond)***
M-PL5					(pH, alkal, cond)***

Full names of metrics are given in Table 1

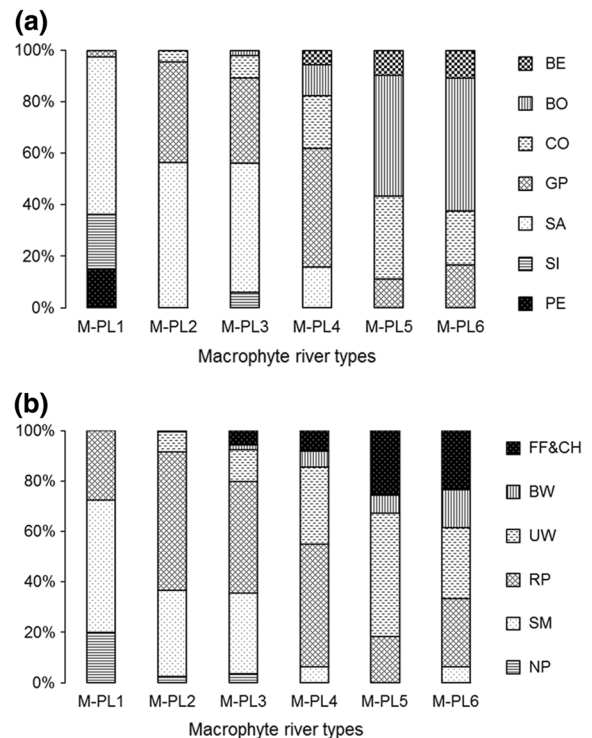
The identified macrophyte types of rivers showed high differentiation in their granulometry of riverbed (Fig. 6a). It was not only the difference between the mountain/upland and lowland rivers. Among the lowland rivers, the differences were found between the humic type (M-PL1) and siliceous rivers (M-M-PL2 and PL3). The humic rivers had a significant fraction of peat and silt in their sediments, whereas siliceous rivers had a significant stony fraction in the substrate. The M-PL2 and M-PL3 rivers were significantly different from the sandy bottom rivers as well as stony streams. The upland rivers (M-PL4) were different from the mountain rivers, which were characterised by a higher proportion of boulders and bedrock.

The flow type differentiation between river types is presented in Fig. 6b. Differences between the lowland and upland/mountain rivers were confirmed. Moreover, the greatest importance of the slowest flow types (imperceptible flow and smooth flow) was detected for the humic rivers, whereas the other types of lowland rivers (M-PL2 and M-PL3) were dominated by ripple flow. The most dynamic types of flow were found in mountain rivers (M-PL5 and M-PL6), where free fall, chute and broken standing waves were recorded regularly.

Discussion

Consistency of the proposed river typology

Based on the macrophyte differentiation, six major river types were identified. The proposed typology

**Fig. 6** Percentage of hydromorphological parameters in separated types of rivers (M-PL1–M-PL6): granulometry of riverbed (a) and flow types (b). Full names of metrics are given in Table 1

applies to most river types identified in Europe, including the system developed during the EU intercalibration exercise for the purpose of the Water Framework Directive (i.e., CIS, 2011; Birk et al., 2006, 2013, 2014). The intercalibration exercise

aimed at introducing consistency and comparability into the classification results of monitoring systems operating in each EU member state for biological quality elements; moreover, uniform water body types were defined in different sub-regions or ecoregions. This typology was quite well associated with our system proposed for the Polish rivers. Our lowland macrophyte river types are relevant to the major EU river types defined for the Central Baltic Rivers, whereas the mountain rivers meet the criteria for the Eastern Continental types and Alpine streams. The rivers that were identified in our study as *Medium and large rivers* (M-PL2) combine two Central Baltic river types: *Medium lowland mixed* (RC4) and *Large lowland mixed* (RC5). *Small lowland rivers* (M-PL3) are relevant to *Small lowland siliceous streams—sand* (RC1) and *Small lowland siliceous—gravel* (RC2). Our *Gravel upland rivers* (M-PL4) correspond to *Small mid-altitude siliceous* (R-C3) Central Baltic rivers as well as the plains: medium-sized, mid-altitude R-E4 Eastern Continental rivers. Our siliceous mountain rivers and upland streams (M-PL7) meet the Carpathian River criteria: small to medium, mid-altitude (R-E1) classification, which is the Eastern Continental type. The R-E1 is limited to an altitude of 800 m, and several M07 rivers were sampled in the higher mountains, therefore, this type of river can also be relevant to the *Alpine—Small to medium, high altitude, siliceous* (R-A2) class with an altitude of 500–1,000 m. A separate group of rivers identified in our study as calcareous mountain, and upland streams (M-PL5) probably corresponds to *Alpine—Small to medium, high altitude calcareous* (R-A1). The estimated altitude of R-A1 was 800–2,500 m, which is higher than most of our sites. Nevertheless, calcareous rivers have thus far not been identified for the Carpathians and East Europe.

The humic river (M-PL1) was already identified for the Polish lowlands (Szozskiewicz et al., 2010a) (named *organic rivers*). Polish humic rivers are most strongly related to the *Small lowland organic rivers* (R-N3) identified for Northern Europe river types (Van de Bund, 2009). Poland was not included in the intercalibration exercise for Northern Europe, which covered the Scandinavian countries as well as the UK and Ireland. Our Polish humic rivers generally do not represent extreme dystrophic conditions with a high content of organic matter and peat, such as in the types of ecosystems in Scandinavia where this is the major

factor identifying *Small lowland organic rivers* (Van de Bund, 2009). Nevertheless, considering the biogeographical conditions (recent glaciation), climate, and land use (UK—Arable: 10%, Permanent crops: 15%, Pasture: 30%, Forests: 30%), as well as flora and fauna defined as a reference of R-N3 (Van de Bund, 2009), we can say that this is very relevant to our M-PL1, which is situated in northern Poland in the wetland and woodland landscape. The extent of peatlands in catchments was also a criterion identifying the peat river type in Ireland (Dodkins et al., 2005).

The analysis of the macrophyte matrix (species and their abundance) using DCA yielded results consistent with the TWINSpan cluster analysis showing the significance of six macrophyte types in the reference conditions. The proposed typology is strongly relevant to the majority of river types identified in Europe for the purpose of the Water Framework Directive (Van de Bund, 2009; Birk & Willby, 2010). The major difference is the uniform group of *Small lowland rivers* (M-PL3), which does not distinguish between stony and sandy river types as was proposed earlier by Szozskiewicz et al. (2010b) and Birk & Willby (2010). Our analysis does not contradict the existence of these two river types, although in the reference conditions of the Polish database, these two groups were not separated according to the macrophyte composition. We discovered strong differentiation in the riverbed substrate, but the significance of the botanical reaction was not observed. Nevertheless, we found that the habitat of lowland reference small streams was determined most strongly by the extensive woody material (fallen trees), and the presence/absence of stones was secondary. This factor determined the rather uniform development of the bryophyte communities of all the small lowland streams. The plant differentiation was observed downstream of the surveyed sites, but these sections were not reference sections according to our strict criteria. For the purpose of monitoring, we recommend that two forms of streams should be distinguished as M-PL3A (*Small lowland rivers—sandy*) and M-PL3B (*Small lowland rivers—gravel*).

Application of the proposed typology across Europe

The conducted analysis included all river types present in Poland. Because the geographical gradient is very wide in Poland, the study represents a major attempt to

cover a significant portion of rivers in Europe. The similarity of macrophyte flora and comparable environmental conditions makes our findings applicable to several other parts of Europe (Holmes, 1983; Baattrup-Petersen et al., 2006, 2008; Birk & Willby, 2010). Our analyses are strongly relevant to four other lowland ecoregions: the Western Plains (13), the Baltic Province (15), Great Britain (18), and Ireland and Northern Ireland (17). Our reference sites found at the moderate altitude may well refer to the Western Highlands (8), whereas sites located in the High Tatra Mountains are to some extent relevant to the Alps (4). In summary, the conducted studies delivered information on the reference sites, which can be applied in the revisions of river typology in the majority of European regions, except for the Mediterranean area and Northern Scandinavia.

Ecological and spatial determination of river typology

We found that the variation of macrophyte communities was determined by several habitat factors, and to a certain extent, by spatial dependence. Each river type, identified by the TWINSpan algorithm and by DCA analysis, could be attributed to a specific complex of habitat conditions, as presented in Fig. 2b. The most important factors are the geological conditions, altitude and the catchment size, as well as the water quality and hydromorphology (bed material and flow type). This typology is strongly relevant to the system included in the EU Water Framework Directive (the European Commission, 2000), which identifies various types according to the size of catchment areas (small, large, medium, very large), geology (calcareous, siliceous, organic) and altitude (high, mid-altitude, lowland).

We have shown that the spatial factor has a rather limited impact on the plant diversity because the identified river types do not accurately reflect the European ecoregion approach (European Commission, 2000). As presented in Fig. 2a, the borderline between the group of lowland ecoregions and the group of ecoregions situated in the highlands and mountains was confirmed by macrophytes. The macrophyte communities do not differ between the studied lowland ecoregions (14 and 16). Such differences cannot be detected between the two upland/mountain ecoregions (9 and 10).

The limited role of the ecoregional pattern in the explanation of the distribution of macrophytes was reported in other studies. For example, Baattrup-Petersen et al. (2006, 2008) conducted a relevant multivariate analysis of macrophyte sites distributed across several European ecoregions. Nevertheless, the importance of the spatial factor was recently emphasised by several studies (e.g., Wiegand, 1984; Demars & Edwards, 2009; Steffena et al., 2014). In these papers, however, the geographical factor was considered on a smaller scale than ecoregions.

Advantages of the analysed dataset

The database of reference lowland rivers included 109 pristine sites and in the case of macrophytes, it was one of the largest datasets analysed to date. Compared with our analysis, the analysis performed by Meilinger et al. (2005) in Germany was based on only 19 reference sites used in the development of a river typology. A larger macrophyte dataset was gathered as a result of the European-wide project STAR, where 64 unimpacted sites (Baattrup-Pedersen et al., 2006) with a strong geographical gradient were surveyed (ten European countries including the Mediterranean area and Scandinavia). This dataset covered lowland and mountain rivers. The relatively large matrix of lowland data were analysed by Baattrup-Petersen et al. (2008). It was a set of 63 reference sites from Northern Europe. The largest set of high quality sites collected thus far was analysed by Birk & Willby (2010), who used EU WFD intercalibration exercise data. It was a set of 111 sites located on rivers in 13 countries in Central and Western Europe and the Baltic region. Compared with our analysis, these databases were less homogenous because the analysed datasets were compiled from different sources, where several survey protocols and large numbers of independent groups of surveyors were involved. The advantage of our dataset is its homogeneity in terms of the strictly standardised field procedure and taxonomical identification. Moreover, all the surveys were performed by a group of surveyors regularly working together, and the process of calibration between them was conducted. The inter-personal source of variability was relatively low compared to other studies, and this type of variability may be an important source of analytical error in botanical studies (Staniszewski et al., 2006). It should also be emphasised that the hydromorphological and water physical and chemical datasets were highly

consistent due to the uniform protocol of the field sampling and laboratory work.

When analysing our large dataset from four different ecoregions, we were able to compare the reference conditions in the wide geographical gradient including lowland, upland and mountain rivers. Our dataset was unique among those analysed thus far because other analyses were limited to lowland sites (Baatrup-Pedersen et al., 2008; Szoszkiewicz et al., 2010a), or the representation of non-lowland sites was negligible (Baatrup-Pedersen et al., 2006). The analysis conducted by Birk and Willby (2010) took into account mountain rivers (in addition to lowland rivers), but only one type of mountain watercourse was considered (small streams in mountains). In this context, our database is exceptionally comprehensive, as it covers all types of watercourses over a wide geographical gradient. Therefore, we were able to develop a comprehensive typology for rivers based on macrophytes.

Reference criteria

The criteria considered for the reference site selection were very strict, and the best quality sites from Poland were selected. Our criteria meet the requirements of the WFD, further explained in the guidelines prepared by the EU Working Group REFCOND involved in the development of the implementation strategy for the Water Framework Directive (Wallin et al., 2003). The range of different elements used to define such conditions included a wide range of parameters related to the catchment land use, hydromorphological characteristics and water quality. Our criteria for selecting the reference sites also generally meet the requirements outlined by other authors (Nijboer et al., 2004; Bald et al., 2005; Meilinger et al., 2005). The thresholds for several physical and chemical components were not as low as in the study of Meilinger et al. (2005) and Pardo et al. (2012) in terms of phosphorous forms and ammonium. This was due to the increased levels of nutrients in some large rivers and humic brooks caused by natural sources. It was a typical pattern of these types of rivers and not a result of anthropogenic degradation. All the affected sites were excluded from the analysis.

Moreover, the increased level of nutrients in our rivers compared to the water quality described in some high quality rivers in other publications (e.g., Pardo et al. 2012) may be due to a different sampling strategy.

The priority of our project was to reach a large number of river sites, and their number reached 1100. To sample high quality sites, we often selected remote and difficult to access locations. Therefore, water analysis was usually limited to one sample collected during the same visit, when plant and hydromorphological surveys were performed. The water quality assessment carried out in our study, therefore, has a more limited value than the analyses conducted during long-term monitoring. It should be noted, however, that the site selection was very strict concerning the human impact, sources of pollution, spatial protection and the other above-mentioned criteria. Samples were not collected during rainy weather or periods with high runoff; instead, if necessary, an additional visit was organised to collect a water sample. A few records with an increased level of certain parameters (the result of natural variability) affected our average values calculated for the different types of rivers. According to our interpretation, the level of water quality presented in our study should be taken into account in lowland Europe when based on individual water samples. More strict criteria (e.g., Pardo et al., 2012) can be recommended, when water quality assessment is based on a long-term central value, which reduces the effect of the temporal natural physical and chemical variability of water.

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